

**NOTES ON THE RESPONSES OF HOST-SEEKING NYMPHS AND ADULTS
OF THE TICKS *IXODES SCAPULARIS* AND *AMBLYOMMA AMERICANUM*
(ACARI: IXODIDAE) TO CANINE, AVIAN, AND
DEER-PRODUCED SUBSTANCES**

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Abstract.—In laboratory and behavioral bioassays, host-seeking nymphs and adults of the blacklegged tick, *Ixodes scapularis* Say, were exposed to substances rubbed from the coats of dogs, from chicken feathers and from the interdigital glands of white-tailed deer, *Odocoileus virginianus* (Zimmermann). Nymphs of the lone star tick, *Amblyomma americanum* (L.) were exposed to substances from chicken feathers. Nymphs of *I. scapularis* became akinetic on surfaces rubbed with substances from chicken feathers, but *A. americanum* nymphs did not. Overall, nymphs of *I. scapularis* appeared to avoid substances rubbed from the coats of dogs. Although hair samples were obtained from 14 dogs maintained under the same conditions, substances from the hair of one dog elicited arrestant responses among nymphs and substances from the hair of another dog appeared to completely repel *I. scapularis* nymphs. Adult *I. scapularis* of both sexes showed high levels of arrestment when exposed to substances from the same canine hair samples. No arrestment was observed when *I. scapularis* nymphs were exposed to interdigital gland substances of white-tailed deer does.

Key Words: kairomones, blacklegged ticks, lone star tick

The host-finding behavior of ixodid (hard) ticks can be divided into two general types, ambushing and hunting (Waladde and Rice 1982). Typically, an ambushing tick waits on or near a vantage point, such as a twig or blade of grass, until a suitable host brushes against the tick's outstretched legs and it catches hold. Ticks that are considered hunters also spend much time waiting for a host, but when they detect the presence of a suitable host from chemical, visual, or other physical stimuli they leave their vantage point or shelter and move toward the source of the stimulus. Many species of ticks employ some of both types of behavior. The host-seeking behavior of the

blacklegged tick, *Ixodes scapularis* Say, tends to be more of the sedentary ambushing sort, whereas lone star ticks, *Amblyomma americanum* (L.), are strongly attracted to carbon dioxide that emanates from hosts during respiration (Wilson et al. 1972) and may be best described as hunters.

Adult ticks of various species, such as *I. scapularis*, *I. neitzi* Clifford, Walker and Keirans, *I. persulcatus* Schulze, *A. americanum*, and *Dermacentor variabilis* (Say) appear to have some ability to select optimum vantage points for ambushing hosts. These adult ticks may locate surfaces (e.g., grass stems) coated with substances rubbed from the pelage or external glands of hosts,

or coated with residues of host urine, and then remain on or near these surfaces (Rechav et al. 1978; Carroll et al. 1995, 1996; Dobrotvorsky et al. 2000). Because many host animals repeatedly use the same trails, feeding and bedding areas, ticks that situate themselves in high host-use locations improve their odds of acquiring a suitable host. Adults of the relatively sedentary *I. scapularis* have been reported to disperse by their own locomotion as far as 10 m over a period of 3–4 wk (Carroll and Schmidtman 1996), and may thereby encounter host-produced kairomonal residues.

In contrast to adult ticks, little is known about whether host-produced chemicals influence where nymphal ticks wait for hosts. The immature stages of *I. scapularis* feed on a wide range of vertebrate species, including birds and lizards (Spielman et al. 1985, Durden and Keirans 1996, Keirans et al. 1996). Although adult *A. americanum*, like adult *I. scapularis*, are strongly associated with white-tailed deer, *O. virginianus* (Zimmerman), their immatures will also parasitize a variety of hosts, and have been reared successfully on domestic chickens (Koch 1983). Little has been reported about nymphal tick attraction to or arrestment on substances from the coats or glands of hosts or to host urine. Arrestment occurs when a tick engaged in ambulatory activity becomes akinetic (Sonenshine et al. 1986) as a behavioral response to certain stimuli (e.g., host-produced kairomone). In laboratory bioassays, nymphs of *I. scapularis* did not show an arrestant response to urine excreted by white-footed mice, *Peromyscus leucopus* (Rafinesque), a principal host of immature blacklegged ticks (Carroll 1999a). However, nymphs of a similar species, the taiga tick, *I. persulcatus*, were attracted to kairomonal constituents in canine hair (Naumov and Vitlin 1995). Nymphs of *A. americanum* are readily attracted to sources of carbon dioxide (Wilson et al. 1972), but their responses to other host-produced chemicals are poorly understood. The purpose of this research was to evalu-

ate some host-produced substances for inducing arrestant activity in nymphs of *I. scapularis* and *A. americanum*, and thereby gain insight as to whether the nymphs of these two medically important species might choose host ambush sites by means of chemical cues.

MATERIALS AND METHODS

Ticks.—Nymphs of *I. scapularis* and *A. americanum* were collected by flagging during May and June in Prince George's County and Queen Anne's County, Maryland. Ticks were maintained at 22°C, 97–99% RH and a photoperiod of 16:8 (L:D) h. Adult *I. scapularis* were collected during the fall in Prince George's County and maintained at 7°C, 97–99% RH and a photoperiod of 11:13 (L:D) h.

Individual ticks used in bioassays were chosen based on their physical integrity (no missing appendages) and motility when removed from storage vials.

Substances.—To obtain samples of substances associated with avian hosts, feathers were removed from 2 male 3-wk old white leghorn chickens. Feathers were removed from the back and wings of the chickens, placed in sealed plastic bags and stored at –15°C. Canine coat substances were obtained from hair clipped from the flanks of 14 mature dogs (4 male, 10 female) kept under uniform conditions and fed identical diets by Auburn University School of Veterinary Medicine, Auburn, Alabama. The hair samples were placed directly in sealed plastic bags, which were kept at –15°C. The interdigital glands of white-tailed deer are located between the toes of the deer, and their secretions are deposited on the substrate and leaf litter. Interdigital gland samples were obtained from hunter-killed deer at a hunter check-in station in Anne Arundel County, Maryland. The hind legs of deer were removed just above the tarsal joint, sealed in plastic bags and frozen at –15°C.

Bioassays.—A 1 µl micropipette was inserted vertically in each end of a rectan-

Table 1. Numbers of *I. scapularis* and *A. americanum* nymphs on micropipettes rubbed with chicken feather substances 24 h after nymphs were released on clay islands with treated and untreated micropipettes^a.

	Treatment	Control	χ^2	<i>P</i>
<i>I. scapularis</i>	19	6	6.76	<0.05
<i>A. americanum</i>	13	11	0.16	>0.05

^aThirty nymphs of each species of tick were tested against mixed feather samples from chickens.

gular clay block (2.5 cm long, 1 cm wide, 1 cm high) centered in a plastic petri dish (3.5 cm diameter, 1 cm high) containing water. The petri dish with the micropipettes was placed in a larger petri dish (9 cm diameter, 1 cm high) containing water, which was in turn placed in a transparent Plexiglas glove box (65 by 85 by 45 cm) containing water 0.5 cm deep. The water confined ticks to the clay islands and provided a high relative humidity ($\approx 95\%$) in the glove box. Capillary tubes (1 mm diameter, 10 cm high) were substituted for the micropipettes when adult ticks were tested.

Feathers or canine hair samples were rubbed between the thumb and forefingers of a vinyl-gloved hand, after which the apical half of one micropipette was rubbed between the gloved fingers. The micropipette at the opposite end of the clay island was rubbed similarly between the thumb and the forefinger of a clean glove. When adult *I. scapularis* were tested, only the apical 1 cm of the capillary tube was rubbed.

About 5 min after interdigital gland samples were removed from the freezer, they were warmed with a hair dryer for ≈ 1 min. Glandular substances were transferred by rubbing the thumb and forefinger of a vinyl-gloved hand between the toes of the leg sample and then on the basal half of a micropipette. The basal rather than the apical portion of the micropipettes was treated in the interdigital gland bioassays, because in nature interdigital gland substances are deposited on the substrate.

A nymph was released singly on the midpoint of a clay island. The location of the nymph was recorded at 1, 18 and 24 h after release. Ticks that were found to have fallen

or crawled into the water during the 2 h after release or overnight were replaced on midpoints of the clay islands. Clay island and petri dish units were washed in soapy water and rinsed with clean water after every test. Thirty *I. scapularis* and 30 *A. americanum* nymphs were tested against feather substances. Ten *I. scapularis* nymphs were tested against hair substances from each of 14 dogs. An additional 10 to 20 *I. scapularis* nymphs were tested against hair substances from 2 dogs that produced extreme results in the initial tests. To confirm kairomonal activity in the canine samples (Carroll 1999b, Dobrotvorskyy et al. 2000) and to compare the behaviors with nymphal responses, 10 female and 10 male *I. scapularis* were tested against substances from 10 of the same hair samples tested against nymphs. Some samples were tested against adults first and others against nymphs first. Thirty *I. scapularis* nymphs were tested against interdigital gland substances from each of 2 white-tailed deer does.

Data were analyzed by chi-square 2×2 contingency tables where responses of each tick were considered independent.

RESULTS

After their release on the clay islands, nymphs of both species tested against substances from feathers, interdigital glands and canine hair wandered up and down the micropipettes. At 1 h after their release, nymphs were often still active and showed no tendency to be on either the treated or untreated micropipettes. By the next morning (18 h after release), nymphs were largely akinetic. Numbers of *I. scapularis* that

Table 2. Numbers of *I. scapularis* on micropipettes (or capillary tubes for adults) rubbed with dog hair substances 24 h after the ticks were released on clay islands with the treated and untreated micropipettes.

	<i>n</i>	Treatment	Control	χ^2	<i>P</i>
nymphs	140	44	78	9.5	<0.05 ^a
male	100	70 ^b	15	35.6	<0.05
female	100	86	7	67.2	<0.05

^a Differed from expected equal distribution between treatment and control, indicating possible tendency to avoid treatment (canine hair substances).

^b Some ticks remained on clay block or fell or crawled into water moat, so treatment plus control numbers may not equal *n*.

became akinetic on the apical portions of micropipettes treated with substances from feathers were significantly greater ($X^2 = 5.4$, $P < 0.05$) than those on the untreated micropipettes (Table 1). No such arrestant response was observed with nymphs of *A. americanum*, with 13 nymphs on treated micropipettes and 11 on untreated ones. Locations of nymphs at 24 h after release differed little from those at 18 h after release.

Canine hair substances were not observed to elicit arrestment among nymphs of *I. scapularis* when data for the samples from all 14 dogs were pooled (Tables 2–3). Instead there was a significant avoidance ($X^2 = 8.2$, $P < 0.05$) of the treated micropipettes. Samples from 2 dogs produced markedly opposite results. When *I. scapularis* nymphs were tested against hair substances from the hair of dog 9656, 19 of 30 nymphs were on treated micropipettes, whereas 4 of the ticks were on the untreated (control) micropipettes. In contrast, none of the 20 *I. scapularis* nymphs tested against hair substances from dog 9334 were on the treated micropipettes 24 h after their release on the clay island. High levels (75–90%) of both male and female *I. scapularis* showed an arrestant response to substances from the same canine hair samples tested against the

conspecific nymphs (Table 3). Adults responded to substances from the hair of dogs of both sexes.

Nymphs of *I. scapularis* did not exhibit an arrestment response to interdigital gland substances from white-tailed deer does. Thirty nymphs were on treated micropipettes and 23 on untreated micropipettes ($X^2 = 0.77$, $P > 0.05$).

DISCUSSION

The responses of the *I. scapularis* nymphs to the chicken feathers indicate that at least certain host species can elicit arrestment among individuals of this life stage. A variety of bird species are reported to serve as hosts for *I. scapularis* nymphs (Anderson and Magnarelli, 1984, Anderson et al. 1985, Battaly et al. 1987, Durden and Keirans 1996, Keirans et al. 1996). Host-seeking nymphs of *I. scapularis* occur primarily in the leaf litter and forest floor where some species of resident birds regularly forage. Repeated foraging or territorial activity by birds at a location would cause oils and other substances on feathers to rub off on fallen leaves and forest floor debris. Thus, it may reward a host-seeking nymph to remain near feather-associated chemical residues on leaf litter. It may be that arrest-

Table 3. Percentage of *I. scapularis* showing an arrestant response to canine hair samples.

Sex of Dog	Nymphs	Females	Males
female	31% (<i>n</i> = 10) ^a	90% (<i>n</i> = 7)	80% (<i>n</i> = 8)
male	33% (<i>n</i> = 4)	77% (<i>n</i> = 3)	75% (<i>n</i> = 2)

^a *n* = number of hair samples from different dogs that were tested against 10 ticks each.

ment in laboratory bioassays highlights a basic response of ticks to host odors that signal ticks to go no further, as if they were actually on a host. If that were the basic unit of behavior, it would account for improved host ambush site selection. Lone star tick immatures will use avian hosts in nature and have been reared on domesticated chickens in the laboratory (Koch 1983). These findings reveal basic differences in how host-seeking nymphal and adult *I. scapularis* and *A. americanum* respond to host-produced substances. Unlike *I. scapularis*, lone star ticks are equipped with eyes (all host-seeking stages) and a rapid gait suited to a more hunting type of host finding (Waladde and Rice 1982). It is, therefore, not surprising to observe different responses by both species to the same chemical stimuli. The results of the bioassays with feathers show that arrestment induced by host-produced kairomones does occur in *I. scapularis* nymphs. However, the lack of the response by the nymphs to interdigital gland substances differs from adult conspecifics, which were strongly arrested by interdigital gland substances from both sexes of deer (Carroll 2001). Interdigital gland substances are deposited directly by deer on the stratum where host-seeking *I. scapularis* nymphs occur. Most adult *I. scapularis* feed on white-tailed deer, but larvae and nymphs will also feed on deer if given the opportunity. Further investigations, focused on rodent and lizard hosts, are needed to ascertain whether chemically mediated behavior plays an important role in host-finding by *I. scapularis* and *A. americanum* nymphs.

Responses of *I. scapularis* nymphs to canine hair samples showed considerable variation. Against most canine hair samples *I. scapularis* nymphs responded indifferently or avoided them, with little indication of arrestment. However, there was a significant arrestant response to one canine sample and apparently complete avoidance of another sample. In contrast, adult *I. scapularis* of both sexes showed high levels of arrestment to the same canine hair samples in bioas-

says. Any of several explanations may account for this discrepancy. Nymphs of *I. scapularis* may have less need to relocate themselves to areas frequented by particular species of vertebrates than adult ticks and may lack a sophisticated mechanism to exploit host-produced chemical cues. A broad array of hosts from small to large mammals (Main et al. 1982, Durden and Keirans 1996, Keirans et al. 1996), birds and even lizards suffice as hosts for *I. scapularis* nymphs. Nymphs of *I. scapularis* may be most responsive to host species not tested. An unfed nymph starts seeking a host near where it dropped from its larval host, probably within the home range of that host and in an area frequented by that host species. An adult *I. scapularis* starts seeking a host near where it dropped from its nymphal host, which may be a species unsuitable for adult feeding (adults feed on medium to large mammals). Thus, adult ticks appear to benefit more from relocating themselves to where large hosts are. This might be reflected in the difference in mobility between these life stages. Adult *I. scapularis* can actively disperse ≥ 10 m in a few weeks, whereas nymphs of the same species have been reported to move 5 m (Carroll and Schmidtman 1996). In order to exploit chemical cues left on vegetation and the substrate by hosts (Carroll et al. 1996), a tick must have the locomotory capability to find them, and the effort in terms of energy and risk must be commensurate with the tick's chances of acquiring a host. Depending on the degree to which a tick searches for host-produced residues in its microenvironment, such host finding behavior could also be considered a form of hunting, albeit different from a tick rushing upwind in a carbon dioxide gradient emanating from a resting host (Waladde and Rice 1982). Furthermore, we cannot assume that a tick wanders continuously until it finds a residue. Ticks may well wait at random vantage points for a time, and, if no hosts pass by, resume wandering. Hence, host-seeking

ticks are not found just along trails and deer beds.

Perhaps in differently designed bioassays more arrestment may have been observed. In any case more bioassays of substances associated with rodent (e.g., white-footed mice) and lizard (e.g., shinks) hosts are warranted, and the threshold activity levels of host-produced kairomones need to be quantified.

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